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TITLE: Prevention and Treatment of Spontaneous Mammary Carcinoma with Dendritic Tumor Fusion Cell Vaccine

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13. ABSTRACT *(Maximum 200 words)*

In the present study, the prevention of cancer development by vaccination with fusion cells was evaluated in a genetically engineered murine model which develops spontaneous mammary carcinomas. The mice (MMT) were produced by crossing MUC1 transgenic mice with strains (MT) that express oncogene polyoma middle T antigen driven by the mouse mammary tumor virus promoter. All MMT (n=23) and MT (n=31) mice in the control groups developed mammary carcinomas within the age of 10-12 weeks and sacrificed after the appearance of larger tumors. In contrast, immunization with fusion cells in the early (<15 days old, n=18) and late premalignant stage (16-30 days old, n=42) provides 72% and 67% protection at age of 20 weeks, respectively, in MMT mice vaccinated with FC/MMT, and 50% and 41% protection at age of 20 weeks, respectively, in MT mice vaccinated with FC/MT. Those mice survived at least 20 weeks free of tumor grossly and microscopically. Furthermore, the tumor appearance was significantly delayed for those mice developed tumors. The CTL activity was consistent with the in vivo results.

We conclude that immunization with fusion cells in the premalignant stage of mammary carcinoma can prevent or delay the tumor development in a model genetically predisposed to cancer. The findings further advances the notion that fusion cells can induce antitumor immunity against multiple tumor antigens including the oncogene product which transforms the normal cells to cancer cells.

14. SUBJECT TERMS

dendritic tumor fusions, vaccination, transgenic mice, spontaneous mammary carcinoma

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Table of Contents

INTRODUCTION:

In our previous annual report, MUC1 transgenic mice were crossed with strains that express polyoma middle T antigen (MTag) driven by the mouse mammary tumor virus (MMTV-LTR) promoter. The new breeder (MMT mice) developed spontaneous mammary carcinoma. Immunization of MMT mice (N=18) with dendritic/tumor fusion cells (FC/MMT) provided protection in 50% mice. In the experiments conducted in the past year, we increased the number of MMT mice in the study and investigated the mechanistic basis for the antitumor immunity.

BODY:

1. Immunization of MMT mice with FC/MMT prevented or delayed the development of spontaneous breast carcinoma.

The mice (MMT) were produced by crossing MUC1 transgenic mice with strains (MT) that express oncogene polyoma middle T antigen driven by the mouse mammary tumor virus promoter. Groups of mice started to be vaccinated at early stages of tumor development. The immunization was repeated 4 additional times at monthly intervals. The mice were followed up to 5-6 months.

All non-vaccinated MMT (n=23) and MT (n=31) mice in the control groups developed mammary carcinomas within the age of 10-12 weeks old and usually died or had to be sacrificed in 3-4 weeks after the appearance of larger size tumor. In contrast, vaccination with fusion cells in the early (<15 days old, n=18) and late pre-malignant stage (16-30 days old, n=42) provides 72% and 67% protection at age of 15 weeks, respectively, in MMT mice vaccinated with FC/MMT, and 50% and 41% protection at age of 15 weeks, respectively, in MT mice vaccinated with FC/MT. Those mice survived at least 5 months free of tumor grossly and microscopically. Furthermore, the tumor appearance was significantly delayed for those mice developed tumors. These results indicate that prophylactic use of fusion cells can prevent or delay the development of mammary carcinoma in a model with known genetic alteration and prone to breast cancer. The earlier commencement of vaccination provides better protection.

2. Vaccination with fusion cells induced humoral and cellular-mediated antitumor immunity

The antitumor immunity in MMT and MT mice induced by vaccination with fusion cells was measured. To determine the humoral immune response, MT and MMT mice were immunized subcutaneously with $5x10^5$ FC/MMT. The immunization was repeated for four additional times at monthly intervals. PBS injection was used as controls. The mice were sacrificed at multiple time points. The blood was collected and analyzed for the presence of anti-MUC1 antibody by ELISA assay. An anti-MUC1 humoral response was induced in MMT mice immunized with FC/MMT. The anti-MUC1 antibody increased after second to fourth immunization. In contrast, there was no, if any, anti-MUC1 antibody detected in MMT mice injected with PBS. These results indicate that immunization with FC/MUC1 was associated with production of anti-MUC1 antibody in MMT mice.

To assess the induction of CTL against MUC1-positive tumor cells, splenocytes from MMT mice immunized subcutaneous with FC/MMT were collected at multiple time points starting the second month. The standard ^{51}Cr release assays was used to determine the CTL activity against the MMT mammary tumor cells (MUC1-positive). CTL activity against syngeneic MMT

mammary tumor cells, at lessor extent, MT mammary tumor cells (MUC1-negative) was demonstrated in MMT mice immunized with varying times of vaccination and at different age. The CTL activity maintained at 20-30% level even at the end of the experiment. In contrast, there was no, if any, CTL activity in MMT mice injected with PBS. The finding that CTL elicited by immunization with FC/MMT lysed the MT mammary tumor cells indicates that polyclonal CTLs were induced. Collectively, prophylactic use of fusion cells induced humoral and T cell-mediated immunity which is sufficient to prevent or delay the development of breast cancer in predisposed model.

DISCUSSION:

Genetic predisposition play a major role in the breast cancer development. The identification of oncogenes and tumor suppressor genes associated with cancer development provides opportunity for immunologic manipulation to target these gene products, so that the onset of cancer development will be inhibited in genetic altered individual. The advent of geneticall engineered mice with targeted gene mutation which mimic the gene alteration in human cancers provides a powerful tool to study the efficacy of vaccines. The transgenic murine models (MMT) developed by Dr. Gendler et al. express the oncogene polyomavirus middle T antigen (PyMTA) under the transcriptional control of the mouse mammary tumor virus (MMTV) promoter long terminal repeats (LTR) and the human MUC1 in a tissue specific fashion. Although PyMTA is not associated with carcinogenesis in humans, it binds signal transduction proteins such as c-Src family, phosphoinositol 3' kinase (PI3) and ras. These proteins are altered in human cancers. The association with and activation of the tyrosine kinase activity of these signal transduction proteins by PyMTA promote cell growth and/or survival and results in widespread transformation of the mammary epithelium and the rapid production of multifocal mammary adenocarcinomas in 100% of the female mice. The majority of the mice develop metastasis in the lungs. Thus, the MMT mice provide us a better tumor model to study the cancer vaccine.

We have developed a vaccine based on the fusions of DC with tumor cells. The fusion cells express MHC class I and II, costimulatory molecules and tumor-derived peptides. Multiple tumor antigens, known or unidentified, are presented by the fusion cells and polyclonal CTL are induced, thus increasing the probability of tumor killings.

In the ongoing study, we immunized the MMT mice with dendritic cells fused with MMT tumor at varying time points of tumor development. We show that immunization of MMT mice with fusion cells in the early stage of carcinogenesis induces humoral and T cell-mediated immunity which is sufficient to block or delay the tumor development in a model genetically predisposed to cancer. The findings further advances the notion that fusion cells can induce antitumor immunity against multiple tumor antigens including the oncogene product which transforms the normal cells to cancer cells.

CONCLUSIONS:

1. Vaccination with DC/MMT fusion cells is effective to block or delay the development of mammary carcinoma in a model genetically predisposed to breast cancer.
2. Polyclonal CTL induced by vaccination with fusion cells is responsible for the antitumor immunity.

PUBLICATIONS

1. Tanaka, Y., Koido, S., Chen, D., Gendler, S., Kufe, D. and Gong, J. Vaccination with Allogeneic Dendritic Cells Fused to Carcinoma Cells Induces Antitumor Immunity in MUC1 Transgenic Mice. Clini. Immuno., 2001, 101:192-200.
2. Shigeo Koido, Yasuhiro Tanaka, Dongshu Chen and Jianlin Gong. The Kinetics of in Vivo Priming CD4 and CD8 T Cells by Dendritic/Tumor Fusion Cells in MUC1 Transgenic Mice. J. Immuno. 2002, 168:2111-2117.
3. Jianchang Xia, Yasuhiro Tanaka, Shigeo Koido, Sandra J. Gendler and Jianlin Gong. Prophylactic vaccination with dendritic/tumor fusion cells prevents the development of spontaneous mammary carcinoma (Manuscript in preparation).

ABSTRACT

Prevention of Spontaneous Mammary Carcinoma by Fusions of Dendritic Cells with carcinoma cells Jianchang Xia and Jianlin Gong **(Third Era of Hope meeting-2002)**

We have demonstrated that immunization with dendritic/tumor fusion cells confers sufficient antitumor immunity to eliminate established pulmonary metastases. In the present study, the prevention of cancer development by vaccination with fusion cells has been evaluated in a genetically engineered murine model that develops spontaneous mammary carcinomas.

The mice (MMT) were produced by crossing MUC1 transgenic mice with strains (MT) that express oncogene polyoma middle T antigen driven by the mouse mammary tumor virus promoter. We demonstrated that MMT or MT mice develop mammary carcinoma in two stages: (i) premalignant stage from new-born to five-weeks of age; (ii) carcinoma formation stage from six weeks of age or older, followed by metastases. Vaccination of groups of mice commenced at early stages of tumor development. The immunization was repeated four additional times at monthly intervals. The mice were followed for five to six months.

All MMT (n=23) and MT (n=31) mice in the control groups developed mammary carcinomas within fourteen weeks of age and usually died three to four weeks after the appearance of tumor. In contrast, immunization with fusion cells in the early (<15-days old, n=18) and late premalignant stage (16-30-days old, n=42) provided 72% and 67% protection, respectively, in MMT mice, and 50% and 41% protection, respectively, in MT mice. These mice survived at least five months free of tumor as observed grossly and microscopically. Furthermore, the tumor appearance was significantly delayed for those mice that developed tumors. The CTL activity was consistent with the in vivo results. Moreover, the telomerase activity in immunized mice was significantly down-regulated compared with that in control groups.

We conclude that immunization with fusion cells in the premalignant stage of mammary carcinoma can block or delay tumor development in a model genetically predisposed to cancer. The findings further advance the idea that fusion cells can induce antitumor immunity against multiple tumor antigens including the oncogene product that transforms normal cells into cancer cells.

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Principal Investigator: Gong, Jianlin

Detailed Cost Estimate

Principal Investigator (last,first,middle): GONG, Jianlin

Detailed Budget for Initial Budget Period					From	Through
					7/1/2002	6/30/2003
				Dollar Amount Requested (omit cents)		
Personnel						
Name	**Role on Project**	**Type Appt. (Months)**	**Annual Base Salary**	**% Effort on Project**	**Salary Requested**	**Fringe Benefits**	**Totals**
Jianlin Gong, M.D.	Principal Investigator	12	125,000	15%	$18,750	$4,350	$23,100
Xiaokun Zeng, Ph.D	Research Associate	12	35,000	100%	$35,000	$8,120	$43,120
Subtotals >>>>>>>>>>>>>>>>>>>					$53,750	$12,470	$66,220
Consultant Costs (Itemize on Justification Page - 3)							$0
Major Equipment Costs (Itemize on Justification Page - 3)							$0
Materials, Supplies, and Consumables Costs (Itemize by Category on Justification Page - 3)							$5,826
Travel Costs (Itemize on Justification Page - 3)							$1,500
Research-Related Patient Costs (Itemize on Justification Page - 3)							$0
Other Expenses (Itemize by Category on Justification Page - 3)							$5,000
Subtotal Other Direct Costs for Initial Budget Period (Subtotal B) >>>>>>>>>>>>>>>>>>							$78,546
Consortium Costs	Direct Cost (Itemize on Justification Page - 3)						$0
	Indirect Cost (Itemize on Justification Page - 3)						$0
Total Personnel & Other Direct Costs For Initial Budget Period							$78,546
Total Indirect Costs For Initial Budget Period							$48,306
Total Costs For Initial Budget Period							$126,852